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Phytotoxicity of *Tagetes erecta* L. and *Tagetes patula* L. on plant germination and growth

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ABSTRACT

This study evaluated the phytotoxic potential and antioxidant activity of *Tagetes patula* and *Tagetes erecta*, and total phenols and flavonoids of the extracts were quantified. Laboratory bioassays for both pre- and post-emergence were performed in *Lactuca sativa* L. seeds and in the *Allium cepa* seeds test. The antioxidant activity was evaluated by DPPH radical scavenging and reducing power of iron, besides total phenols and flavonoids quantification in the extracts. Thus, was observed a reduction in the mitotic index when in compared with the negative control. Was observed also a reduction the germination and development of tested seedlings and was verified a considerable antioxidant potential and presence of flavonoids and phenolic compounds in the extracts. According to these results, it is possible to conclude that *T. erecta* and *T. patula* have phytotoxic compounds that may enhance and expand their use in the management of organic agriculture, mainly in vegetables.

1. Introduction

Organic agriculture has grown rapidly in recent decades (Ponti et al., 2012), being practiced in different environments and configurations (Oelofse et al., 2010). This system is intended to enhance agricultural production with minimal damage to ecosystems (Seufert et al., 2012), promoting the reduction of toxic residues and providing the production of contaminants free food, coupled with a high nutritional value (Gusmão et al., 2012). Currently, organic agriculture has excelled in some crops, such as in fruits and oil seeds production (Connor, 2013), making it economically competitive in relation to conventional techniques, thereby driving an increasing global demand (Oelofse et al., 2010; Ponti et al., 2012).

Several crops with high economic value are already produced organically (Oelofse et al., 2010), as is the case of maize and sugar cane (Ariedi Junior et al., 2012). Another important crops that stands in organic management are the production of vegetables (Souza, 2011), for example, lettuce, jambu, arugula, kale, cabbage, and cilantro, among others (Gusmão et al., 2012). Among the techniques of organic management, the use of natural or biological origin pesticides has been highlighted, since they have low toxicity, efficient control, reduced cost, are ease to acquire and do not promote resistance to pests and diseases in agricultural crops (Saminêz et al., 2008; Souza, 2011).

The use of species of the *Tagetes* genus in organic agriculture is described in different works (Dietrich et al., 2011), especially in the culture of vegetables due to its bactericidal, nematicidal, fungicide and insecticide action (Vasudevan et al., 1997; Scrivanti et al., 2003; Rondon et al., 2006; Xu et al., 2011; Jain et al., 2012; Dasgupta et al., 2012). Scientific studies have verified that species of this genus have thiophenes (Dasgupta et al., 2012), alkaloids, polyacetylenes, fatty acids (Vasudevan et al., 1997), flavonoids and terpenes, with the last groups being the main compounds with possible antimicrobial and nematicidal effects (Vasudevan et al., 1997; Rondón et al., 2006; Santos, 2013).

Species of the *Tagetes* genus are popularly known in Brazil as marigold and belong to the Asteraceae family. They are native to Mexico and Central America (Peres, 2007; Santos, 2013). Among the species of this genus, *Tagetes patula* points out for being used due to its effect of attractiveness or repellency of insects (Gonçalves and Silva, 2003; Peres., 2007; Silveira et al., 2009). Besides, it is popular for having medicinal properties, such as analgesic, antispasmodic, immune stimulant, laxative and anthelmintic (Duque, 2006; Priyanka et al., 2013) and bactericidal (Rondón et al., 2006; Jain et al., 2012). *Tagetes erecta*

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shows antimicrobial compounds (Jain et al., 2012), nematicide, fungicide and insecticide activity (Xu et al., 2011), being widely used in different syndications of agricultural crops (Duque, 2006; Peres, 2007; Ferraz and Freitas, 2008).

Considering the importance of *Tagetes* species usage in organic agriculture management, mainly in the production of vegetables, as well as the different biological activities attributed to the species of this genus, this study evaluated the allelopathic, cytotoxic and antioxidant potentials of extracts of *T. patula* and *T. erecta*. In addition, it quantified the total phenols and flavonoids of the extracts.

2. Materials and method

2.1. Vegetal material and extract preparation

The vegetative parts of *T. patula* and *T. erecta* were collected from specimens grown in the field in Agência Paulista de Tecnologia dos Agronegócios (APTA) — Polo Regional Médio Paranapanema (22°37′ 07.92″ S and 50°22′26.85′ W, with altitude 701 m). For preparation of extracts, plant parts were washed, dried in an oven (40 °C) and crushed in a knife mill. The aqueous extract was obtained by mechanical agitation in distilled water [1:10 (w/v) for 24 h at room temperature]; after three filtrations the samples were frozen and lyophilized (Model L101, Líotop, Brazil) to obtain the dry extract. The hydroethanolic extract was obtained by mechanical stirring in a solution of ethanol:water (70:30) at a ratio of 1:10 (w/v) for 24 h, and the process was repeated three times with the same plant material. Then, the extract was filtered and rotary evaporated (model MA120, Marconi, Brazil) at 60 °C to remove the ethanol. Subsequently, it was frozen and lyophilized to obtain the dry extract. Similarly, the ethanolic extract was collected by replacing the ethanol:water solution (70:30) for absolute ethanol (Impex, Brazil), being that the dried extract was obtained after concentration on a rotary evaporator followed by drying at room temperature.

2.2. Determination of osmotic potential, pH and electrical conductivity

The osmotic potential was determined according to the technique described by Villela et al. (1991). The treatment was evaluated by osmotic solutions obtained using polyethylene glycol 6000 (PEG6000). The values of osmotic potential obtained in PEG6000 solutions were compared with the values found in the different concentrations of the extracts of *T. patula* and *T. erecta*.

The pH from the different extract concentrations of *T. patula* and *T. erecta* was determined using a pH meter (Tecnopon ® model: MPA210). Similarly, the electrical conductivity was measured with a conductivity meter (Conductivity Meter Instrutherm®, model: CD860).

2.3. Bioassay of allelopathy for pre-emergence

The pre-emergence bioassay was conducted with seeds of *Lactuca sativa* L. cv. Grand Rapids (lettuce) by controlling the germination of these plants in Petri dishes (60 mm × 15 mm) and germination paper with relative humidity, temperature and light artificially was controlled in greenhouse of Germination type BOD (Biological Oxygen Demand) (model: 411/FPD, New Ethics, Brazil). This experiment was set up in a completely randomized design (CRD), where the Petri dishes were divided into experimental and control groups. Each plate contained 50 seeds of lettuce, with six replicates for each experimental group treated with different extracts of *T. patula* and *T. erecta* (at concentrations of 5, 10 and 20 $mg \cdot mL^{-1}$) and a negative control group (water). The protrusion and geotropic curvature of the radicle was used as germination criteria, as indicated by Labouriau (1983). The seeds that showed false germination by soaking were not accounted for in the results. The germination of the species was monitored every 6 h over 48 h. From the resulting data obtained in the assay, different indices were calculated: germinability or germination percentage ($[\sum ni / A] \cdot 100$), germination mean time ($Tm = [\sum ni \cdot ti] / \sum ni$), and germination mean speed ($Vm = 1/Tm$) and germination synchronous ($U = -\sum_{i=1}^{k} [fi \log 2fi]$), in which ni = the number of seeds that germinated in each time gap "ti"; A = the total number of seeds in the test; and ti = the time gap between the beginning of the experiment and the observation time (Santana e Ranal, 2004; Pereira et al., 2009).

After bioassay pre-emergence, we washed the seeds that did not germinate with distilled water and were maintained in a greenhouse for germination BOD.

2.4. Bioassay of allelopathy for post-emergence

We conducted the bioassay according to the methodology proposed by Soares and Vieira (2000) and Alves et al. (2004) and adapted it to our laboratory conditions. Lettuce seeds were previously germinated in Petri dishes lined with germination paper moistened with distilled water. After 24 h under BOD greenhouse conditions, the seedlings that showed an average of 2 mm in length were used in the bioassay. The latter, was set up in a completely randomized design (CRD) with Petri dishes containing germination paper moistened with 1 mL of the solution from the different extract concentrations of *T. patula* and *T. erecta* at concentrations of 5, 10 and 20 $mg \cdot mL^{-1}$. We divided these into experimental and control groups, containing 25 seedlings on each plate with four replicates per treatment and for the control (water).

We observed the evolution process of the treatments and the measurement of roots and hypocotyls were performed using a digital caliper (model: IP65, DIGIMESS®, Brazil) every 24 h up to 48 h of exposure (Miró et al., 1998; Procópio et al., 2005).

2.5. Statistical analysis for pre- and post-emergence testing

For statistical treatment of pre- and post-emergence tests, normality (Shapiro–Wilks) and homogeneity tests (Levene) were conducted. The data did not present normality, and its variances were not homogeneous, therefore, we analyzed the results using the Kruskal–Wallis and Dunn test ($\alpha = 0.05$) with the use of BioEstat 5.3 software, according to the model proposed by Santana and Ranal (2004).

2.6. Evaluation of antioxidant activity

2.6.1. Stable DPPH free radical scavenging activity

The stable 1.1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA) radical scavenging activity was determined by Blois's method (1958). The extracts of each sample were dissolved in absolute ethanol different concentrations (250, 500, 1000, 2000, and 4000 $\mu g \cdot mL^{-1}$) and then mixed with 250 μL solution of DPPH (500 μM). The extracts reacted with the DPPH radical for a period of 30 min at a low luminosity. Then they were submitted to the UV–vis spectrophotometer (Femto-600 Plus) at a 517 nm wave length. The calculation of the antioxidant activity was performed according to the formula: antioxidant activity (%) = [(control − sample) / control] × 100. The effective concentration and quantity of antioxidant required to decrease the initial concentration of DPPH by 50% (EC50) was estimated from an exponential curve is obtained by plotting on the abscissa the concentration of the sample ($mg \cdot mL^{-1}$), or positive control and the ordinate, the percentage of antioxidant activity. Gallic acid (Vetec-Química Fina, Brazil) was used as the reference. Three repetitions were undertaken.

2.6.2. Ferric ion reducing antioxidant power (FRAP assay)

FRAP activity was measured according to the method of Rufino et al. (2006). Briefly, acetate buffer (300 mM, pH 3.6), TPTZ (2.4.6-tripyridyl-s-triazine) 10 mM in 40 mM HCl and $FeCl_3 \cdot 6H_2O$ (20 mM) were mixed in the ratio of 10:1:1 (v/v/v) to obtain the FRAP reagent. The reagent (2.7 mL), ultrapure water (270 μL) and sample solutions (90 μL) were mixed thoroughly. Next we kept it in a water bath (Cienlab® model EC 400/4) for 30 min at 37 °C. The absorbance was measured at